

BIOAVAILABILITY OF POLYCYCLIC AROMATIC HYDROCARBONS IN FIELD-CONTAMINATED ANACOSTIA RIVER (WASHINGTON, DC) SEDIMENT

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Abstract—Sediment–water partitioning behavior and bioavailability of five polycyclic aromatic hydrocarbons (PAHs; phenanthrene, pyrene, chrysene, benzo[k]fluoranthene, and benzo[a]pyrene) were measured in field-contaminated sediment collected from moderately polluted regions of the Anacostia River (Washington, DC, USA). Much of the sediment PAH burden was resistant to desorption: Effective partition coefficients were 2- to 10-fold greater than expected from literature values, and more than 80% of PAHs remained sorbed after treatment of the sediment with a nonionic polymeric adsorbent (Amberlite XAD-2) for 20 h. Bioaccumulation, elimination, and assimilation of each PAH in the deposit-feeding tubificid oligochaete *Ilyodrilus templetoni* were measured and compared with the equivalent measurements from laboratory-inoculated sediment. *Ilyodrilus templetoni* effectively accessed the desorption-resistant fraction of these organic contaminants, as exhibited by high single-gut passage assimilation efficiencies (ASEs) of the five PAHs (60% < ASE < 90%). However, steady-state accumulations of PAHs by *I. templetoni* were very low and consistent with low pore-water concentrations. The present results suggest that steady-state accumulation of PAHs is controlled by pore-water concentrations and is not necessarily related to route of uptake or assimilation efficiencies.

Keywords—Bioavailability Biota–sediment accumulation factor Desorption resistant Oligochaete worm

INTRODUCTION

Sediments serve as the ultimate sink for many hydrophobic organic contaminants (HOCs), such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls. Sediments also represent biologically important environmental habitats. Information regarding the availability of the sediment-associated HOCs to sediment-dwelling organisms, such as deposit-feeding invertebrates, is crucial for understanding contaminant transfer into both these organisms and consumers higher up the food chain. Bioaccumulation measurements often are used to determine the bioavailability of sediment-associated contaminants and trophic transfer potential of contaminants in aquatic environments [1]. Bioaccumulation or net uptake of a HOC often is indicated by the biota–sediment accumulation factor (BSAF), which is defined as the ratio of lipid-normalized tissue concentration over the organic carbon-normalized sediment concentration [2]:

$$\text{BSAF} = \frac{C_i/f_{\text{lipid}}}{C_s/f_{\text{OC}}} \quad (1)$$

where C_s is the sediment concentration of HOCs ($\mu\text{g/g}$ dry wt), C_i is the tissue concentration of HOCs ($\mu\text{g/g}$ dry wt), f_{OC} is the fraction of total organic carbon content of the sediment (weight fraction, %), and f_{lipid} is the fraction of the tissue lipid content (weight fraction, %). This definition is based on the equilibrium partitioning theory [3, 4], which assumes that the HOCs distribute between sediment organic carbon, pore water, and lipids of biota and that, at equilibrium, partitioning among these three phases is reproducible.

The equilibrium partitioning theory predicts that BSAF will be close to unity if an organic contaminant partitions similarly

between the lipid of an organism and the organic carbon fraction of the sediment. Biota–sediment accumulation factors of approximately one have been demonstrated by Ankley et al. [5] for both laboratory and field populations of benthic oligochaetes exposed to a wide variety of polychlorinated biphenyls and by Ingersoll et al. [6] as well as Gewurtz et al. [7] for PAHs. However, significant deviations from unity also have been observed as a result of chemical transformation, biomagnification, or differential partitioning potential between lipids and sediment organic carbon [8–10]. In addition, BSAFs of significantly less than one have been observed for sediments in which the fast-desorbing fraction significantly decreased as a result of contaminant sequestration or removal of the fast-desorbing fraction by adsorbents [11–13].

Our previous studies concerning the bioavailability of phenanthrene and benzo[a]pyrene in laboratory-inoculated sediment demonstrated that observed reductions in BSAF were approximately proportional to increases in the sediment–water partition coefficient (i.e., proportional to decreases in the pore-water concentration) associated with the desorption-resistant fraction. Sediment pore-water concentration, controlled by physicochemical desorption processes, determined the equilibrium tissue concentration of the tested contaminants independent of the route of uptake and assimilation efficiency [14, 15]. This suggests that physicochemical measurements (i.e., pore-water concentration or observed sediment–water partition coefficient) will effectively predict bioavailability. Because use of the pore-water paradigm to define bioavailability has been limited to laboratory-inoculated sediments in the previous work [14,15], testing of this model in field-contaminated sediments is desired. Few studies have been conducted to determine the relationship between pore-water concentrations and uptake in field-contaminated sediments as a result of complications associated with sequestration, mass-transport limita-

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Table 1. Sediment-water partition coefficients (K_{oc}) in field-collected and laboratory inoculated sediments^a

	PHE	PYR	CHR	BkF	BaP
Sediment concentration ($\mu\text{g/g}$ dry wt)	1.67 (0.17)	4.64 (0.90)	1.54 (0.37)	0.74 (0.06)	1.57 (0.17)
Field-collected log K_{oc}					
Without correction for DOC	4.74 (0.06)	5.38 (0.03)	5.85 (0.07)	6.48 (0.20)	6.51 (0.03)
With correction for DOC	4.76 (0.06)	5.43 (0.03)	6.05 (0.07)	6.74 (0.20)	6.79 (0.03)
Laboratory-inoculated log K_{oc}					
Rapidly desorbing	4.32 (0.03)				6.21 (0.06)
Desorption resistant	4.82 (0.06)				6.26 (0.06)
Expected log K_{oc}	4.36	4.97	5.65	5.79	5.83

^a Values in parentheses represent the standard deviation. BaP = benzo[a]pyrene; BkF = benzo[k]fluoranthene; CHR = chrysene; DOC = dissolved organic carbon; PAH = phenanthrene; PYR = pyrene.

tions, strong binding to soot, and variability in the polarity of organic matter [16]. Although other researchers have proposed pore-water concentration as a good predictor of bioaccumulation [4,17,18], to our knowledge simultaneous observations of physicochemical availability by experiments regarding desorption as well as biological uptake rate and extent have not been conducted with field-contaminated sediments.

The primary objectives of the present study were to determine if pore-water concentrations in field-contaminated sediments define the extent of accumulation in a deposit-feeding invertebrate and to determine if bioaccumulation is independent of the contaminant rate of uptake and single-gut passage assimilation efficiency.

MATERIALS AND METHODS

Sediment selection and PAH quantification

Sediment employed in the present study was collected during the year 2002 from the Anacostia River, which flows from Maryland (USA) to Washington, DC, and the Potomac River. Major contaminants in the sediment include PAHs, polychlorinated biphenyls, and metals. Here, we focus on the bioavailability of PAHs. Total PAHs in the collected sediment averaged approximately 30 $\mu\text{g/g}$, and total organic carbon content averaged $4.32\% \pm 0.16\%$ (mean \pm standard deviation). The sediment was passed through a 2-mm sieve to remove debris and large particles and then homogenized for the experiments.

An ultrasonic extraction method based on standard U.S. Environmental Protection Agency Method 3550 [19] was used to extract PAHs from sediment (and, later, from worm tissue). The extract was analyzed by a Hewlett-Packard 1100 series high-performance liquid chromatograph with ultraviolet-diode array (Hewlett-Packard, Palo Alto, CA, USA) and fluorescence detectors. The procedure includes grinding sediment or tissue with sodium sulfate, followed by addition of a solvent (sediment was extracted with hexane and acetone [1:1, v/v], and tissue was extracted with dichloromethane) and then sonication for 25 min. For samples of worm tissue, the extracts were cleaned with a silica gel column based on U.S. Environmental Protection Agency Method 3630C [20]. Ultimately, the solvent was exchanged with acetonitrile before analysis with high-performance liquid chromatography.

Five of the 16 PAHs in the sediment samples—phenanthrene, pyrene, chrysene, benzo[k]fluoranthene, and benzo[a]pyrene—were identified with high confidence for the present study. These five PAHs exhibit a wide range of hydrophobicity ($4.5 < \log K_{ow} < 6.5$). Table 1 lists the measured

concentrations of the five selected PAHs in Anacostia River sediment.

Desorption measurement

Physicochemical availability of PAHs was assessed by measurement of the sediment–water partition coefficient and by evaluation of the fraction that would rapidly desorb after treatment with a nonionic polymeric adsorbent, Amberlite XAD-2 (divinylbenzene-styrene copolymer; Supelco, Bellefonte, PA, USA). Pore-water concentration of the tested sediment, as indicated by sediment–water partition coefficients, were measured using standard procedures of the American Society for Testing and Materials [21], as we have employed previously [14,15] but with minor modifications. For each sample, 2.5 g of sediment and 140 ml of electrolyte solution (0.01 M NaCl, 0.01 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 0.01 M NaN_3 in deionized water) was used to achieve recommended solid to water ratios in the desorption vials. The sediment slurry was tumbled for 20 d to allow partitioning to reach apparent equilibrium and then centrifuged at 700 g for 20 min to separate water from the sediment. Although the water to solid ratio of these measurements is much higher than the water to solid ratio in the sediments, pore-water concentrations were expected to be effectively identical at equilibrium because of the small mass fraction of PAHs in the water (typically $<1\%$ for all PAHs of interest except phenanthrene, which was expected to be $<5\%$). In situ measurement of the pore-water concentration using diffusion samplers (peepers) also were considered, but heterogeneity suggests that it would not necessarily better represent pore-water concentrations of the collected, homogenized sediment.

Eighty milliliters of the derived pore water was extracted with 4 ml of dichloromethane, and another 10 ml of the water were measured for dissolved organic carbon (DOC) content in the water using a TOC-5000A total organic carbon analyzer (Shimadzu, Columbia, MD, USA). An additional water sample was directly injected into a high-performance liquid chromatograph, but concentrations were too low for detection for most PAHs of interest. Both truly dissolved and DOC-associated HOCs likely are extracted by dichloromethane. Therefore, extraction by dichloromethane overestimates the free or true pore-water concentration, and the water concentration derived by solvent extraction was corrected for DOC using the following equation:

$$C_w = \frac{C_{wm}}{1 + C_{DOC}K_{DOC}} \quad (2)$$

where C_{wm} is the dissolved water concentration (mg/L), C_w is the free or truly dissolved water concentration (mg/L), C_{DOC} is the DOC content in water, and K_{DOC} is the DOC–water partition coefficient of HOCs. Large uncertainties exist in the determinations of K_{DOC} because of the limitations in measuring the freely dissolved water concentration. For the present study, the correlation of Burkhard [22] between K_{DOC} and K_{OW} based on PAH data from different DOC sources and different analytical methods was employed:

$$\log K_{OC} = \log K_{OW} - 0.58 \quad (3)$$

The equilibrated sediment concentration (C_s , $\mu\text{g/g}$) was calculated based on mass balance. The observed partition coefficient (K_{OC}^{obs}) was calculated according to a linear partitioning model [23]:

$$K_{OC}^{obs} = \frac{K_p}{f_{OC}} = \frac{C_s}{C_w f_{OC}} \quad (4)$$

where K_p is the sediment–water partition coefficient of the HOC (L/kg). Although the correction for partitioning to DOC is subject to large error, the maximum influence on K_{OC}^{obs} was 0.28 log units (for the most hydrophobic compound, benzo[a]pyrene).

The fast-desorbing fraction of PAHs was evaluated by short exposure (20 h) to a nonionic polymeric adsorbent, Amberlite XAD-2. Because of its strong affinity for aromatic compounds, Amberlite XAD-2 has been used in cleaning up water samples with trace PAHs [24] and in assisting desorption of PAHs from field-contaminated sediment [25]. The Amberlite XAD-2 was purchased from Supelco and has a particle size of 20 to 60 mesh and a surface area of 300 m^2/g . Wet sediment (100–200 g; moisture content, $\sim 45\%$) was mixed thoroughly with 10% (dry-wt ratio) XAD-2 and stored for 20 h, which was demonstrated by Chai [26] to be appropriate for characterizing the fast-desorbing fraction of contaminant from sediment based on the shape of the desorption kinetics by XAD-2. At the end of exposure, sediment and XAD-2 were separated, and chemical concentrations in sediment and XAD-2 resin were determined using the method described above. The fast-desorbing fraction was defined as the fraction of contaminant in sediment removed by XAD-2.

Bioavailability studies

The freshwater deposit-feeding tubificid oligochaete *Ilyodrilus templetoni* (Southern) was used in the present bioavailability studies, as in our previous studies with laboratory-inoculated sediments. Sexually mature *I. templetoni* had an average wet weight of 3.2 mg and a dry weight to wet weight ratio of approximately 0.17 to 1 on initial collection from the culture.

Bioaccumulation as well as assimilation efficiency and elimination rate were measured in a manner employed previously [14,15]. Bioaccumulation (*I. templetoni* tissue concentration) of PAHs from Anacostia River sediment was measured at 5, 10, 15, 20, and 26 d to estimate the kinetics of uptake and the time to reach steady state. Each chamber was considered as a replicate measure of bioaccumulation, and four replicates were used in each exposure period. Lipid-normalized tissue concentrations at different exposures were analyzed by one-way analysis of variance. Differences were considered to be statistically significant at $p < 0.05$.

Assimilation efficiency was measured using the pulse-chase feeding technique [27] and was based on direct measurement

of the contaminant concentration ingested after 40 min (time for one gut passage or less) exposure to field-collected Anacostia River sediment and the concentration remaining in tissue after complete egestion (depuration in clean sediment for 4 h), which is the contaminant assimilated into the tissue. Assimilation efficiency was calculated as the ratio of the contaminant assimilated over the total contaminant ingested. In the elimination rate measurement, after exposing worms to Anacostia River sediment for 14 d to achieve sufficient body burden of PAHs, worms were then exposed to sediment without PAHs and allowed to eliminate assimilated contaminants. The clean sediment used during the elimination period was a lower-organic-carbon sediment (1.2% organic carbon) from Bayou Manchac (LA, USA). The lower organic carbon content and other factors may cause the elimination rate in this sediment to be different than would have occurred in the Anacostia River sediment, but direct measurement of elimination rates in the contaminated Anacostia River sediment was not possible. The quantity and rate of elimination was determined by collection and analysis of whole-worm tissues at 0, 6, 12, 24, 48, and 72 h. Tissue-concentration data were fit to a first-order decay model (Eqn. 5), from which the elimination rate constant (k_e) was determined:

$$C(t) = C(0) \cdot e^{(-k_e t)} \quad (5)$$

where t represents time (h).

RESULTS AND DISCUSSION

Comparison of equilibrium partitioning in field-contaminated sediment with laboratory-inoculated sediment and calculated values

Measured K_{OC} values of PAHs in field-contaminated sediment are shown in Table 1, along with the previously measured K_{OC} values in laboratory-inoculated sediments [14,15] and K_{OC} values predicted from K_{OW} [23] (Eqn. 6). Table 1 also includes estimated partition coefficients with and without a correction for association with DOC:

$$\log K_{OC} = \log K_{OW} - 0.218 \quad (6)$$

The measured partition coefficients for all five PAHs in the Anacostia River sediment were significantly higher (2- to 10-fold) than expected by the predictive relationship (Eqn. 6) of Karickhoff et al. [23]. The fast-desorbing fraction as measured by XAD-2 desorption was effectively 0 to 22%, illustrating large amounts of contaminants remaining on the sediment and in a desorption-resistant fraction. Chai [26] showed that 29% of the organic matter in the Anacostia River sediment remained after 24-h combustion at 375°C. This nonvolatile organic carbon measured in this manner has been used as an indicator of condensed-phase carbon exhibiting greater sorption capacity and slow desorption kinetics. The effective partition coefficient of benzo[a]pyrene in the Anacostia River sediment was almost one order of magnitude higher than the literature partition coefficient and those measured in short-term, laboratory-inoculated sediment by Lu et al. [15].

Uptake, assimilation, and elimination

Figure 1 shows the uptake of phenanthrene, pyrene, chrysene, benzo[k]fluoranthene, and benzo[a]pyrene by *I. templetoni* from Anacostia River sediment during the 26-d exposure in 50-ml test tubes. Tissue concentrations of all compounds except phenanthrene were essentially constant after 5 d of exposure. The analysis of variance showed no significant

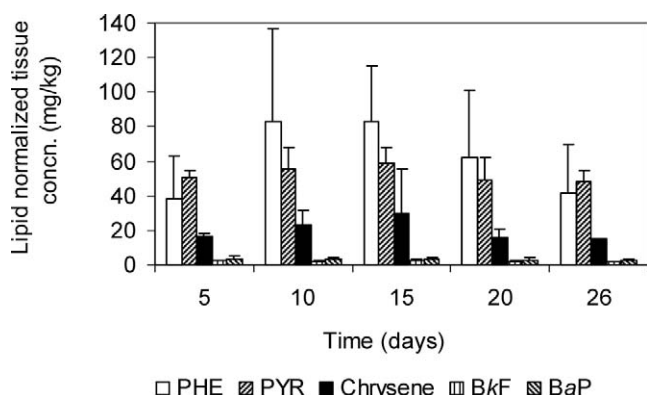


Fig. 1. Uptake of phenanthrene (PHE), pyrene (PYR), chrysene (CHR), benzo[k]fluoranthene (BkF), and benzo[a]pyrene (BaP) by *Ilyodrilus templetoni* in field-collected Anacostia River (Washington, DC) sediment. Error bars represent one standard deviation from the mean.

changes over time in lipid-normalized accumulation for any of the selected PAHs (all $p > 0.05$), suggesting that a steady-state tissue concentration was achieved within 5 d. Although similar rapid achievement of steady-state conditions was observed with phenanthrene in laboratory-inoculated sediment [14], benzo[a]pyrene kinetics in laboratory-inoculated sediment was much slower [15].

Single-gut passage assimilation efficiencies of PAHs in this field sediment were very high, ranging from 63 to 88% across compounds (Table 2). Assimilation efficiencies of phenanthrene and benzo[a]pyrene were approximately equal for field-collected and laboratory-inoculated sediments (Table 2). The high assimilation efficiencies indicate that even contaminants exhibiting desorption resistance were effectively accessed by this deposit feeder.

Although assimilation efficiencies for contaminants from field-collected and laboratory-inoculated sediments were similar, elimination rates (after depuration) from field-collected sediment were significantly higher (by approximately an order of magnitude). The higher elimination rates may have been caused by the organism response to the different sediments (Bayou Manchac) in which elimination was measured. Because the microbial community presumably was acclimated and self-selected for the Anacostia River sediments, PAH degradation was much faster than in the laboratory-inoculated sediment experiments. High microbial activity and PAH degradation rates were shown in the Anacostia River sediment compared to Bayou Manchac sediment (M. Nabatilan, Louisiana State University, Baton Rouge, LA, USA; unpublished data).

Bioaccumulation and applicability of pore-water paradigm

The BSAFs in the 50-ml test tubes were calculated at each exposure period in which tissue concentrations were measured. The BSAFs calculated from the measured lipid-normalized concentration for all five PAHs are listed in Table 3. These BSAFs were compared with those derived from experiments conducted previously with laboratory-inoculated sediments [14,15,28]. With the exception of that for phenanthrene, measured BSAFs were significantly lower ($p < 0.001$) in field-contaminated Anacostia River sediment than in laboratory-inoculated sediments. As indicated above, steady state also was rapidly achieved compared with that in laboratory-inoculated sediments and may be the result of the comparatively low normalized accumulation in Anacostia River sediment, whereas assimilation efficiencies between the two sediments were equivalent. Similar results were observed by Van holf [29] when comparing the time to reach steady state for “aged” sediment with that for freshly inoculated sediment. Phenanthrene exhibited anomalously high BSAFs and a high variability that was not observed in the other PAHs. As the least hydrophobic and most volatile of the compounds evaluated, phenanthrene was the least subject to desorption-resistant phenomena and the most subject to both biotic and abiotic fate processes, potentially complicating analysis and evaluation of results.

Low BSAFs (i.e., <1) of the more hydrophobic compounds are consistent with the high desorption-resistant fraction of the contaminants, as indicated by the high effective partition coefficients or low pore-water concentrations measured in the sediment. Our previous studies regarding laboratory-inoculated sediment showed that reductions in BSAF were approximately proportional to increases in sediment–water partition coefficients as the desorption-resistant fraction increased [14, 15]. As pore-water concentrations of PAHs decreased because of increases in sediment–water partitioning, the corresponding steady-state tissue concentration also decreased, regardless of the route of exposure (either absorption from pore water or ingestion of sediment [30]). This behavior can be expressed by the model that Lu et al. [14] defined:

$$\text{BSAF}^{\text{res}} = \frac{K_{\text{OC}}^{\text{lab}}}{K_{\text{OC}}^{\text{res}}} \text{BSAF}^{\text{lab}} \quad (7)$$

where BSAF^{res} is the effective BSAF as a result of exposure to sediments containing the desorption-resistant fraction of contamination, BSAF^{lab} is the BSAF if the contaminants were purely in the labile fraction, $K_{\text{OC}}^{\text{res}}$ is the organic carbon–normalized sediment–water partition coefficient of the desorption-resistant fraction, and $K_{\text{OC}}^{\text{lab}}$ is the organic carbon–normalized sediment–water partition coefficient of the labile fraction. The

Table 2. Assimilation efficiencies (ASE) and elimination rates (k_e) by *Ilyodrilus templetoni*^a

Sediments	PHE	PYR	CHR	BkF	BaP
Field collected					
ASE (%)	62.6 (7.0)	77.3 (12.2)	81.5 (13.4)	75.2 (3.9)	88.1 (17.2)
k_e (/h)	0.22	0.058	0.040	0.047	0.064
Laboratory inoculated					
ASE (%)	50.0				80.4
k_e (/h)	0.042				0.0066

^a Values in parentheses represent the standard deviation. See Table 1 for definitions of abbreviations.

Table 3. Biota-sediment accumulation factors (BSAFs) of polycyclic aromatic hydrocarbons in Anacostia River (Washington, DC, USA) sediment^a

	PHE	PYR	CHR	BkF	BaP
Measured BSAFs ^b	1.55 (1.04)	0.49 (0.09)	0.48 (0.11)	0.11 (0.04)	0.09 (0.03)
Predicted BSAFs ^c	0.40 (0.35–0.46)	0.34 (0.32–0.37)	0.40 (0.34–0.47)	0.11 (0.07–0.18)	0.11 (0.10–0.12)

^a See Table 1 for definitions of abbreviations.

^b Values in parentheses represent the standard deviation.

^c Values in parentheses represent predicted values based on one standard deviation from the average of observed sediment-water partition coefficients.

K_{OC}^{lab} was predicted from K_{OW} (e.g., that defined by Eqn. 6), whereas the K_{OC}^{res} was measured directly. Using this model and assuming that $BSAF^{lab}$ equals unity, the BSAFs of PAHs in field-contaminated Anacostia River sediment were predicted and compared with the experimentally measured BSAFs, as shown in Table 3. The predicted BSAFs (excluding the highly variable phenanthrene data) exhibited a high correlation with the measured BSAFs ($r^2 = 0.90$), and the predicted BSAFs typically are less than one standard deviation from the measured values, which implies that the effective partition coefficient or pore-water concentration is a good predictor for the BSAF of PAHs in the field-collected Anacostia River sediment. A comparison of measured BSAFs and predictions of $BSAF^{res}$ based on $BSAF^{lab}$ equals unity, measured pore-water concentration data from previous studies in the literature [14,15,28,31], and those measured herein are plotted in Figure 2. This further supports the conclusion of Lu et al. [14,15] that pore-water concentrations ultimately control the bioaccumulation of PAHs (as a model of HOCs) in the deposit-feeding organisms. Steady-state BSAF proved to be unrelated to assimilation efficiency, suggesting that extraction by digestive fluids [32, 33] will not predict bioaccumulation under these conditions. This conclusion was reached previously with laboratory-inoculated sediment [14, 15, 17], but the present results provide further support with field-contaminated sediment.

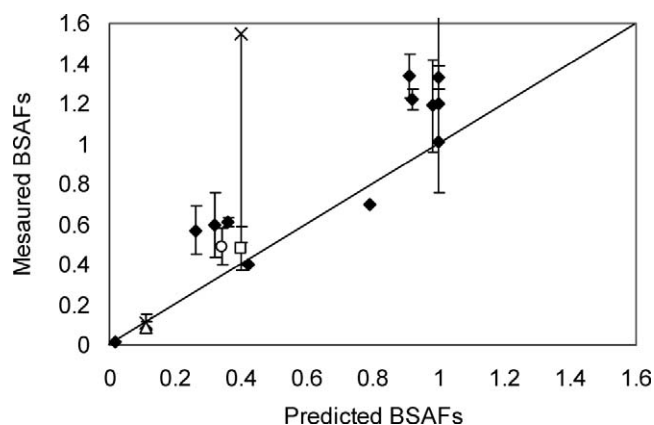


Fig. 2. Experimentally measured biota-sediment accumulation factors (BSAFs) versus BSAFs predicted from the measured effective partition coefficient (Eqn. 7) assuming that $BSAF^{lab}$ (the BSAF if the contaminants were purely in the labile fraction) equals unity. Error bars represent one standard deviation from the mean (data from Millward et al. [28] were calculated values without replicates). ♦ = laboratory-inoculated sediments from previous studies in the literature [14, 15, 28, 31], and open symbols represent field-contaminated sediment from the present study (× = phenanthrene; ○ = pyrene; □ = chrysene; * = benzo[k]fluoranthene; △ = benzo[a]pyrene).

CONCLUSION

Field-collected Anacostia River sediment exhibited a lower pore-water concentration of PAHs, as reflected by a higher sediment-water partition coefficient and correspondingly lower BSAFs compared to laboratory-inoculated sediment; however, the assimilation efficiencies of these sediments were very close. The present results further support the conclusion that physicochemical measurements of pore-water concentration can be used to predict the bioavailability and bioaccumulation potential of sediment-associated PAHs, which had been demonstrated previously by laboratory-inoculated sediment [14,15,17]. Sediment-quality criteria often are defined on the basis of equilibrium partitioning from the solid phase [34]. The present data suggest that a more effective means of defining exposure and risk from PAH-contaminated sediments is from measured pore-water concentrations. Measured pore-water concentrations reflect the fate processes of contaminants in pore water and any dynamic desorption limitations associated with the desorption-resistant fraction of contaminants while still indicating directly the steady-state uptake in the deposit-feeding organisms in these studies.

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